Emirates Journal of Food and Agriculture. 2023. 35(6): 511-519
doi: 10.9755/ejfa.2023.v35.i6.3117
http://www.ejfa.me/

RESEARCH ARTICLE

Current status and molecular phylogeny of some economically important viruses infecting wheat crops of Şırnak province, Turkey

Orhan Kapan[1], Mustafa Usta[1*], Abdullah Güller[2]

[1]Van Yuzuncu Yil University, Faculty of Agriculture, Plant Protection Dept, Van/Turkey, [2]Bingöl University, Faculty of Agriculture, Plant Protection Dept, Bingöl/Turkey

ABSTRACT

A survey of yellow dwarf viruses (YDVs) -associated diseases of wheat plants was performed for the first time in the Şırnak Province, Turkey. In plants wheat cultivated areas, symptoms such as diminished leaf size, dwarfing, yellowing, and reddening of leaves wheat plants were observed in cultivated areas. A total of 441 specimens were collected regardless of showing symptoms and assayed for Barley/Cereal Yellow Dwarf Viruses [B/CYDV] association by PCR tests using virus coat protein gene (CPG)-specific primer pairs. These assays demonstrated that 13.38% (55 samples) were positive to the occurrence of BYDV-PAV (9.73%) and CYDV-RPV (0.73%) for wheat viruses, but no BYDV-MAV, -SGV, and -RMV infections of BYDVs were found in PCR assays of collected samples. In addition, double infection of BYDV-PAV and CYDV-RPV was detected in 12 samples with a 2.91% infection rate. The CPG sequences of randomly selected two isolates were revealed using bacterial cloning and sequencing. Sequences obtained were deposited in GenBank under Accession numbers OL685734 and OL685736 for BYDV-PAV; and OL685735 and OL685737 for CYDV-RPV. BLASTn analysis of CPG sequences of four isolates shared high nucleotide homology with their intraspecies isolates, with 97.35-98.67% for two BYDV-PAV and 97.72-97.89 for two CYDV-RPV. The consensus tree constructed classified B/CYDV-associated wheat viruses with their phylogenetically closest individuals from Turkey and the world. This survey is the first report of BYDV-PAV and CYDV-RPV associated with wheat plants in Şırnak Province of Turkey.

Keywords: BYDVs; Coat protein gene; Incidence; Sequencing; Şırnak

INTRODUCTION

Wheat crop is a critical and highly adaptable cultivated plant that plays a strategic role in human and animal consumption worldwide (Shewry, 2009). With 3.5% of the world's wheat area, Turkey has an annual wheat production amount of about 20 million tons. The annual production amount for Şırnak Province of Turkey is 153 thousand tons (TUIK, 2020). Wheat agriculture is one of the primary food sources worldwide, but many bacterial, fungal, and viral diseases limit wheat production, resulting in reduced yield and quality (Gomes et al., 2016). The impact of virus diseases on wheat plants is particularly severe in developing countries. Therefore, due to the lack of effective control measures, it is highly important to conduct research focused on identifying, diagnosing, managing wheat virus diseases, and minimizing the resulting product losses (Kılıç et al., 2012). In world grain and wheat production areas, BYDVs are prevalent viral agents, causing economic damage to wheat at rates ranging from 5% to 25% (Wiese, 1987; Kennedy and Connery, 2005).

The causative agents of the disease are infectious for plants belonging to the Poaceae family, such as wheat, barley, oats, corn, etc., and it causes a decrease in grass yield in over 150 hosts from meadow and pasture plants (Gould and Shaw, 1983; İlbağı, 2017). Wheat viruses are usually diagnosed based on the symptoms and signs observed in plants. These symptoms include yellowing of leaves, striped or mosaic appearance, leaf curling, stunting of plant growth, reduction in plant height, reduced heading, and decrease in grain yield. However, since these symptoms can be caused by many different diseases, laboratory tests are essential to make an accurate diagnosis (Irwin and Thresh, 1992; Hoffman and Kolb, 1997). Laboratory tests are typically performed using immunological tests such as

***Corresponding author:**
Mustafa Usta, Van Yuzuncu Yil Unversity, Plant Protection Deparment, Turkey. **E-mail:** mustafausta@yyu.edu.tr.

Received: 30 August 2022; **Accepted:** 12 March 2023

ELISA (Enzyme-Linked Immunosorbent Assay) (Deligöz et al., 2011) and molecular tests (PCR, RT-PCR) (Kwon et al., 2014). These tests detect the presence or absence of viruses based on their antigenic properties or genetic structures. In addition, plant samples exhibiting disease symptoms can be collected and examined using imaging techniques such as electron microscopy. By examining the characteristic structures and properties of viruses using these methods, a diagnosis can be made (Shepard and Carroll, 1967). Viral transmission between hosts occurs in a persistent mod by vectors of 25 aphid species, but not by egg, physical routes, and seed (Thackraya et al., 2009; Deligöz et al., 2011; Abraha, 2020).

Wheat viruses are widespread and the prevalence of these viruses varies depending on geographic location, climate, and agricultural practices. Wheat plant hosts 55 different viruses, including *Wheat streak mosaic virus* (WSMV), *Wheat dwarf virus* (WDV), *Barley yellow dwarf virus*-PAV, BYDV-MAV, BYDV-RMV, BYDV-SGV, *Cereal yellow dwarf virus*-RPV, and *Barley stripe mosaic hordeivirus* (BSMV) (Erkan and Yılmaz, 2009). BYDVs consist of 9 different genetic variants: BYDV-PAV, BYDV-MAV, BYDV-SGV, BYDV-RMV, BYDV-GPV, BYDV-PAS, BYDV-GAV, and CYDV-RPV (İlbağı 2017). Viral diseases related to B/CYDV were reported in distinct agroecosystems at varying infection incidences, such as Tunisia, Poland, Pakistan, Yemen, Latvia, USA, England, New Zealand, South-Eastern Australia (D'Arcy et al., 1992; Kendall et al., 1996; Bisnieks et al., 2004; Kumari et al., 2006; Delmiglio et al., 2010; Siddiqui et al., 2012; Trzmiel, 2019; Nancarrow et al., 2021).

In Turkey, viral infections of wheat plants have been reported in various locations (Köklü, 2004; Ilbagi et al., 2005, 2008; Ilbagi, 2006), but there was no information on wheat-associated BYDVs diseases for Şırnak Province of Turkey. Results of this performed to elucidate the prevalence and etiology of the YDV disease of wheat (BYDV-PAV, -MAV, -SGV, -RMV, and CYDV-RPV) in this region are submitted here. Additionally, another aim of this study is to uncover the phylogenetic relationships of the identified viral isolates with other isolates worldwide using gene-based programs.

MATERIAL AND METHODS

Sampling and inspecting

The survey area was calculated to cover at least 3% of the total production area of the surveyed region utilising the cascade sampling method (Bora and Karaca, 1970). To confirm the possible presence of BYDVs, 411 fresh leaf samples were gathered in distinct regions of Şırnak Province in the April and May seasons of 2020. Regardless of simptom development, five leaf samples representing each surveyed area (Köklü, 2004) were brought to the virology laboratory of Van Yuzuncu Yıl University in cold chain and stored at -20 °C until total RNA extraction. The number of samples collected within the borders of Şırnak Province is 45 in Center, 200 in İdil, 110 in Silopi, 31 in Cizre, 10 in Güçlükonak, 10 in Uludere, and 5 in Beytüşşebap.

Total RNA extractions and complementary DNA synthesis

Total RNA was extracted from young frozen wheat leaves using the silica-based method described by Foissac et al. (2001). The reverse transcription process cDNA was performed using reverse primers (YAN-R primer for detection and gene-specific primers for characterization) and cDNA synthesis kit (Fermentas, Germany) according to manufacturer instructions.

Oligonucleotide primer pairs for BYDVs

The primers used in enhanced and multiplex PCR tests were got from previous studies and synthesized by Sentebiolab (Ankara/Turkey). In enhanced and multiplex PCR assays, subgroup-specific (subgroup I and II) and CPG-specific primers were used (Table 1).

Enhanced and multiplex PCR tests to determine single or the mixed infections

To selectively reveal the possible BYDV subgroups, SHU-F, S2A-F, and S2B-F together with YAN-R were included in the same reaction in Multiplex PCR assays. Enhanced-PCR tests were performed to determine the presence and ratios of BYDVs subgroup members in the samples giving positive reactions in BYDV subgroup discrimination using primers given in Table 1. Multiplex PCR program composed of 94°C for 5 min (initial denaturation), 35 cycles of denaturation 30 s at 94°C for 30 s, annealing 60°C for 30 s, and elongation 72°C for 30 s, and final elongation 72°C for 7 min. PCR master mix was prepared in 25 µl volume including 2.5 µl of cDNA template, 0.2 µl of Taq DNA polymerase (Fermentas, Germany), 17.3 µl RNase free water, 1.5 µl $MgCl_2$, 0.5 µl dNTPs, 2.5 µl 10×Buffer, and 0.5 µl subgroup-specific primers. Enhanced-PCR parameters were the same as above, and the PCR duration was used as described in Table 2. The PCR cycling conditions in enhanced PCRs are 35 cycles for BYDV-MAV and -RMV, and 40 cycles for BYDV-PAV, -SGV, and CYDV-RPV in denaturation, annealing, and elongation steps.

PCR amplicons and DNA marker (1kb) were verified in 0.9% an EtBr-added agarose gel in 1X TBE buffer and monitored in a gel imaging system (Synoptic Ltd. Cambridge, GB). Isolates from preserved previously and DNA-free master mix were employed as positive and negative controls, respectively.

Table 1: Primer data employed in PCR assays for detected of YDVs in wheat crops

B/CYDVs	Name	Sequences	Amplicon	References
Subgroup 1 (BYDV-PAV, MAV, SGV)	YAN-R SHU-F	TGTTGAGGAGTCTACCTATTTG TACGGTAAGTGCCCAACTCC	832 bp	Malmstrom and Shu, 2004
Subgroup 2 (CYDV-RPV, BYDV- RMV)	S2A-F S2B-F	TCACCTTCGGGCCGTCTCTATCAG TCACCTTCGGGGCGTCTCTTTCTG	372 bp	
BYDV-PAV	PAV F PAV R	ACCTAGACGCGCAAATCAAA ATTGTGAAGGAATTAATGTA	590 bp	
BYDV-MAV	MAV R MAV F	CGGATCAGGTTTGGGCTCTG ATGAATTCAGTAGGCCGTAG	660 bp	Bisniek et al., 2004
BYDV-SGV	SGV F SGV R	ACCAGATCTTAGCCGGGTTT CTGGACGTCGACCATTTCTT	237 bp	Deb and Anderson, 2008
BYDV-RMV	RMV F RMV R	GACGAGGACGACGACCAAGTGGA GCCATACTCCACCTCCGATT	365 bp	

Table 2: Chart showing denaturation, annealing, and elongation temperatures and times used in enhanced PCR tests for the detection of individual wheat viruses

Isolates	Predenaturation		Denaturation		Annealing		Elongation	
BYDV-PAV	94°C 1 cycle	10 min	94°C	30 s	42°C (30 s)	72°C	30 s
BYDV-MAV		2 min		30 s	53°C (30 s)		90 s
BYDV-RMV		2 min		30 s	58°C (1 min)		30 s
CYDV-RPV		2 min		30 s	53°C (1 min)		30 s
BYDV-SGV		10 dk		30 s	55°C (30 s)		30 s

Cloning, sequencing, phylogenetic relationships

Two PCR-positive amplicons that were detected for viruses were randomly selected for further characterization through cloning and sequencing. For this, the complete CP genes of viral isolates were amplified using CPG-specific primer pairs which were 'F-ATGAATTCAGTAGGTCGTAG' and 'R-GAGGAGTCTACCTATTTGGC' for BYDV-PAV, and 'F- ATGAGTACGGTCGTCCTTAG' and 'R- CCCTATTTTGGGTTTTGTAGC' for CYDV-RPV (Usta et al., 2020). PCR conditions, units, and cycles were set as described above for BYDV-PAV and CYDV-RPV, except for the annealing temperature of 44 °C of CYDV.

PCR-amplified yields were inserted into pGEM T-Easy vector (Promega, Madison, Wisconsin, USA) and transformed into *E. coli* competent bacteria strain JM109 (Promega, USA). Viral sequences-inserted positive clones were also confirmed by colony PCR tests. Recombinant plasmids were isolated from transformed bacteria using a purification kit (GeneJET Plasmid MiniPrep Kit, Thermo Scientific, USA) and sequenced using New Generation System by a relevant company (Sentebiolab, Turkey). Present DNA sequences were recorded in GenBank. To elucidate nucleotide identity, the CPG sequences obtained were analyzed by BLASTn program in NCBI GenBank (http://www.ncbi.nlm.nih.gov). To cluster the sequences, the phylogenetic relationships based on CPG amplicons were created by CLC Main Workbench 6.7.1 software using sequences from various agro-geographic origins and hosts, with included outsource for each BYDVs.

RESULTS AND DISCUSSION

Symptoms in suspicious wheat plants

BYDVs adversely affect grain crops by reducing yield and quality all over the world (Perry et al., 2000; Rastgou et al., 2005), and infection by BYDV naturally can lead to 11-13% yield loss (Miller and Rasochová, 1997). Depending on the period of infection and the wheat variety, BYDVs produce noticeable common symptoms such as chlorosis, late heading, leaf reddening, and stunting in cereal crops (Yount et al., 1985; İlbağı et al., 2006). Similar to those previously reported, we observed symptoms evoke of BYDVs disease such as wheat plants with reddening at the leaf tips, dwarfed plants, yellowish lines on the leaves, and yellowed or dried patches in the fields surveyed in Şırnak Province in 2020 (Fig. 1). However, abiotic factors such as drought and salt stress, nitrogen and potassium deficiency can also partially mimic the viral disease symptoms of wheat plants (Jain et al., 2013; Diaz et al., 2006; Zhang et al., 2020). We molecularly tested samples of wheat to confirm the presence of viral RNA.

Infection incidences of BYDVs using by multiplex RT-PCR and enhanced RT-PCR methods

To detect the viral genome, molecular approaches were employed by various researchers. Compared to serological methods like ELISA, PCR-derived methods are helpful because of their high sensitivity and time consuming (Steyer et al., 2005). Multiplex PCR assays were preferred by numerous plant virologists to define simultaneously multiple virus infections in cultivated

crops (Nie and Singh, 2000; Saade et al., 2000). Concurrent amplification of plant-pathogen viruses, viroids, and phytoplasma by multiplex PCR assays was reported in sweet potato and potato (Kwak et al., 2014; Cating et al., 2015), in stone fruits (Sánchez-Navarro et al., 2005) in wheat (Tao et al., 2012; Hassan et al., 2018), in tobacco (Günay and Usta, 2020), in grapevine (Gambino and Gribaudo, 2006), in cucurbits (Kwon et al., 2014), in chrysanthemum (Zhao et al., 2015), in barley and triticale (Trzmiel, 2017), in the citrus tree (Roy et al., 2005), and in garlic (Park et al., 2005).

In the present study, group-specific primers in Multiplex PCR tests revealed that the wheat samples were infected with at least one subgroup virus of BYDVs, amplifying 832bp and 372bp DNA fragments (Fig. 2). Enhanced PCR tests achieved using virus-specific primer pairs were confirmed the BYDV-PAV and CYDV-PPV in infected wheat samples in Şırnak province of Turkey.

In enhanced PCR tests, virus-specific primer pairs generated the DNA bands of 603 bp for BYDV-PAV and 615 bp for CYDV-RPV (data not shown). The master mix devoid of DNA was PCR-negative. No BYDV-MAV, -SGV, -RMV positivity were detected in any collected samples. Of the 411 samples, 55 had a positive reaction for BYDVs with a general infection rate of 13.38% based on the enhanced RT-PCR results. In addition, double infection of BYDV-PAV+CYDV-RPV was found in 12 samples with a 2.91% infection rate. Region-based detailed infection incidences are summarized in Table 3.

Fig 1. Typical redness and yellowed leaf tips of wheat plants characterized by BYDVs diseases.

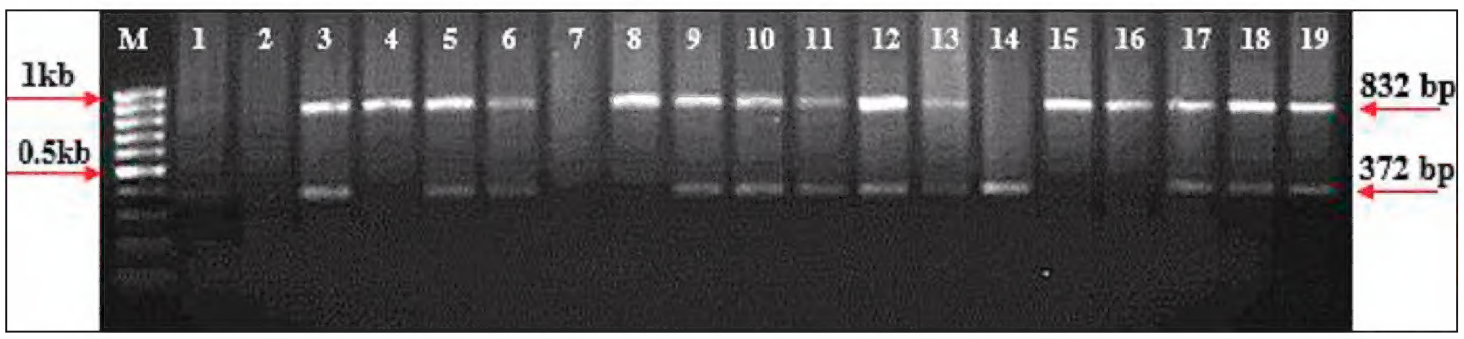

Fig 2. Agarose gel image showing the BYDVs-positivity from wheat samples. DNA band sizes of 832bp and 372 bp indicated the presence of at least one GroupI (BYDV-PAV, -MAV, -SGV) and GroupII (BYDV-RMV and CYDV-RPV). M: 1kb DNA ladder (Thermo Scientific),1: Positive control, 2: Negative control, and Others: Multiplex PCR-positive samples of BYDVs.

Table 3: Chart showing the prevalence of mix and single infections of B/CYDVs in wheat plants from Şırnak Province of Turkey

Surveyed Counties	Tested Samples	BYDV-PAV		CYDV-RPV		PAV+RPV		Incidence (%)	
		IP	PI	IP	PI	IP	PI	IP	PI
İdil	200	19	9.5	1	0.5	-	-	20	10
Silopi	110	15	13.63	1	0.90	12	10.90	28	25.45
Center	45	2	4.44	1	2.22	-	-	3	6.66
Cizre	31	1	3.22	-	-	-	-	1	3.22
Güçlükonak	10	3	30	-	-	-	-	3	30
Uludere	10	-	-	-	-	-	-	-	
Beytuşşebap	5	-	-	-	-	-	-	-	-
Total	411	40	9.73	3	0.73	12	2.91	55	13.38

*IP: Number of infected wheat plants in the collected samples, PI: Percentage of infected wheat plants in the total sample (%).

The wheat-associated viral disease were well-studied thorough the world and the sustainability of wheat farming is threatened by numerous viruses (Gitton et al., 2002; Usta et al., 2020; Kumar et al., 2020). BYDVs are the most common group of wheat studied worldwide and reported their prevalence with different percentages using serological or molecular tools. Various BYDVs subgroups reported that BYDV-PAV in China and Hungary (Khine et al., 2020; Áy et al., 2008), -PAV, -MAV, -SGV, -RMV, and CYDV-RPV, in Argentina, Ethiopia, and Pakistan (Webby et al., 1993; Bashir et al., 1997; Abraha, 2020), -PAV, -MAV, -SGV in Brazil (Parizoto et al., 2013), -PAV and –MAV in Tunisia (Hamdi et al., 2020), -PAS in the Czech Republic (Kundu, 2008), -GAV and -GPV in China (Li et al., 2015). BYDV-PAV was confirmed to be the most common virus in wheat plants tested against BYDVs worldwide (Conti et al., 1990; El-Yamani and Hill, 1990). The present work elucidated that BYDV-PAV has a high frequency compared to other YDV agents among 411 wheat samples, with a 9.73% infection incidence. These outputs harmony with research conducted around the world. In this study, BYDV-MAV, -SGV, -RMV were not found in wheat samples of field surveys of Şırnak Province, Turkey. However, presence frequency and infections of these viruses were reported in wheat plants from various districts surveyed of Turkey (Pocsai et al., 2003; Ilbagi et al., 2008; Deligoz et al., 2011; Hassan et al., 2018; Kılıç et al., 2019; Usta et al., 2020). With 0.73%, CYDV-RPV had the lowest disease potential compared to BYDV-PAV. The occurrence of this virus in Turkey has been reported by various researchers at a low frequency in wheat crops compared with other studies related to BYDVs (Usta et al., 2020; Hassan et al., 2018; Köklü, 2004; Karaozan and Usta, 2020; Erler, 2019).

Molecular characterization of CPG of Şırnak isolates

Among the B/CYDV-positive isolates, two B/CYDV-positive isolates were randomly selected, and their CPG segments were amplified using specific primers. The amplification was successful (Fig. 3), and the resulting DNA sequences were cloned and analyzed. The Şırnak isolates produced 603bp and 615bp DNA sequences for BYDV-PAV and CYDV-RPV, respectively. Sequences obtained were submitted in GenBank under Accession number OL685734 (Şırnak 1) and OL685736 (Şırnak 4) for BYDV-PAV, and OL685737 (Şırnak 33) and OL685735 (Şırnak 12) for CYDV-RPV.

BLASTn program employed to identify the nucleotide sequence similarity revealed that Şırnak BYDV isolates are in a close evolutionary relationship with those in different parts of the world, with minor nucleotide differences. Multiple sequence comparisons executed using the CLC Main Workbench 6.7.1 program ascertained the nucleotide sequence differences in Şırnak sequences. Sequence characterization data of Turkish-Şırnak isolates were submitted in Table 4.

Molecular Genom Phylogeny of BYDV-PAV and CYDV-RPV Şırnak Isolates

Based on sequences obtained of BYDV-PAV and CYDV-RPV isolates, consensus trees were constructed with world

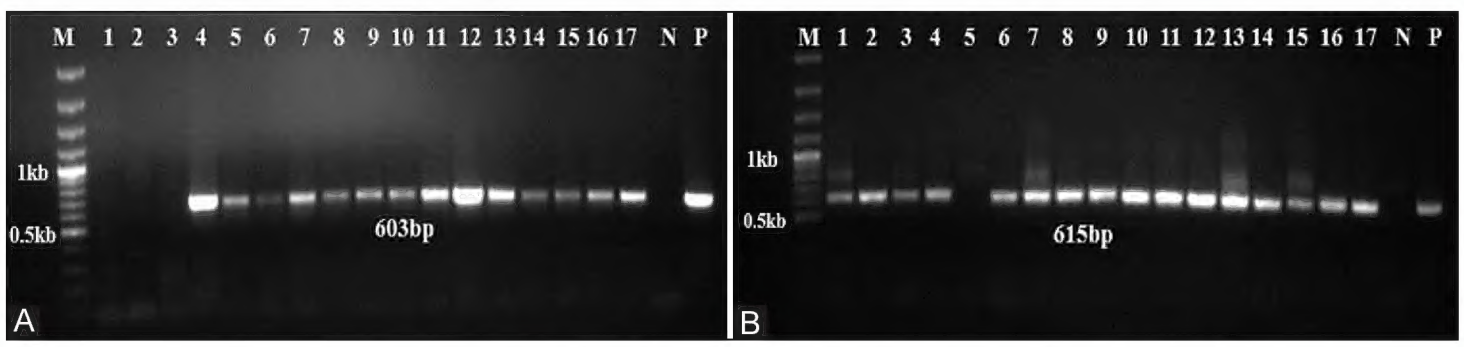

Fig 3. Agarose gel image showing the amplified coat protein gene of BYDV-PAV (A) and CYDV-RPV (B) from wheat samples. M: 1kb DNA marker, P: Positive control, N: Negative control.

Table 4: BLASTn analysis outputs and nucleoid substitutions of Turkey-Şırnak isolates

Virus	Isolate	Identity (%)	Relationship	Substitutions (%)	Nucleotide (bp)
BYDV-PAV	Şırnak 1	89.72	Australia Pakistan USA	7.8 (47 nt)	603
		98.67	Mardin/Turkey		
	Şırnak 4	89.88	Australia Pakistan Netherlands	3.48 (21 nt)	
		97.35	Mardin/Turkey		
CYDV-RPV	Şırnak 12	44.88	New Zealand	1.95 (12nt)	615
		97.89	Mardin/Turkey		
	Şırnak 33	44.72	New Zealand	2.6 (16 nt)	
		97.72	Şırnak/Turkey		

isolates from distinct hosts, including the *Zucchini yellow mosaic virus* outgroup (JF792368). A total of 18 isolates for BYDV-PAV and CYDV-RPV were chosen from previously defined worldwide (Table 5).

According to the consensus tree of BYDV-PAV, Şırnak 1 (OL685734) and Şırnak 4 (OL685736) Turkish isolates basically clustered with other Turkish isolates detected in wheat from Mardin province (MK732034) (Fig. 4). In addition, other Turkish isolates (KX774424 and KC900900) were in the same group from wheat in Diyarbakır and Van province, due to probably highly conserved CP gene sequence similarity and the same origin.

Based on the consensus tree of CYDV-RPV, Şırnak 12 (OL685735) and Şırnak 33 (OL685737) Turkish isolates exhibited a close phylogenetic relationship with other Turkish isolates (MK732035 and KC900903) from Van and Mardin province of Turkey and clustered together (Fig. 5).

In this study, the frequency of wheat plants infected by BYDVs is relatively low, as in other areas of the Eastern

Table 5: Chart showing the CPG-specific sequence information and hosts of BYDV-PAV and CYDV-RPV in the GenBank

BYDV-PAV isolates			CYDV-RPV isolates		
Acc. Nos	**Host**	**Origin**	**Acc. Nos**	**Host**	**Origin**
JQ811489	*Zea mays*	Pakistan	DQ988087	*Avena sativa*	ABD
DQ285671	-	ABD	DQ115527	-	ABD
M21347	-	Australia	HM488009	*T. aestivum*	Pakistan
KY634904	*Hordeum vulgare*	England	DQ988108	*H. vulgare*	ABD
KT198976	*Triticum aestivum*	Pakistan	DQ988088	*H. vulgare*	ABD
KY634899	*H. vulgare*	China	AY450425	-	İran
KY634901	*H. vulgare*	France	GU002338	*Avena sativa*	N. Zealand
KY634886	*Lolium multiflorum*	Holland	DQ988093	*H. vulgare*	ABD
KT198985	*T. aestivum*	Pakistan	DQ988105	*A. sativa*	ABD
JX067825	-	Brazil	KY564215	*L. multiflorum*	Germany
KP096696	*T. aestivum*	Hungary	KY564214	*L. multiflorum*	Germany
KY634912	*T. aestivum*	Sweden	KY564213	*L. multiflorum*	Germany
KY634911	*H. vulgare*	Czech Rep.	EF408187	*Dactylis glomerata*	N. Zealand
KY634896	*T. aestivum*	Germany	DQ115534	*A. fatua*	ABD
KP096694	*T. aestivum*	China	JX294312	*A. sativa*	Azerbaijan
KC900900	*T. aestivum*	Turkey	KY634929	*Poa annua*	Germany
MK732034	*T. aestivum*	Turkey	KC900903	*T. aestivum*	Turkey
KX774424	*T. aestivum*	Turkey	MK732035	*T. aestivum*	Turkey

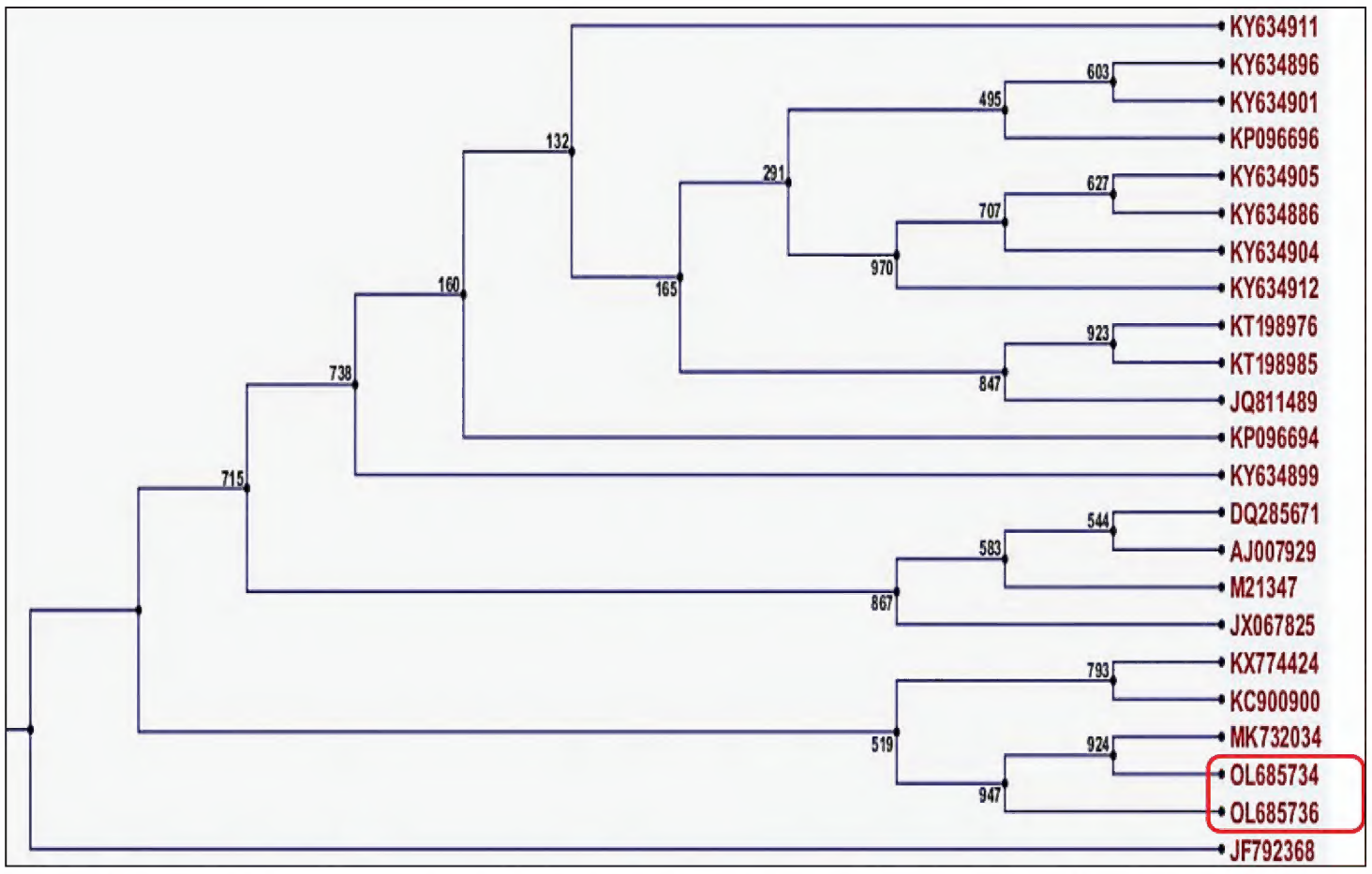

Fig 4. Consensus tree created by Neighbor-Joining Method using 1000 bootstrap values of CPG of Şırnak 1 and Şırnak 4 BYDV-PAV isolates along with isolates from other origins. Turkey-Şırnak isolates are in the red circle.

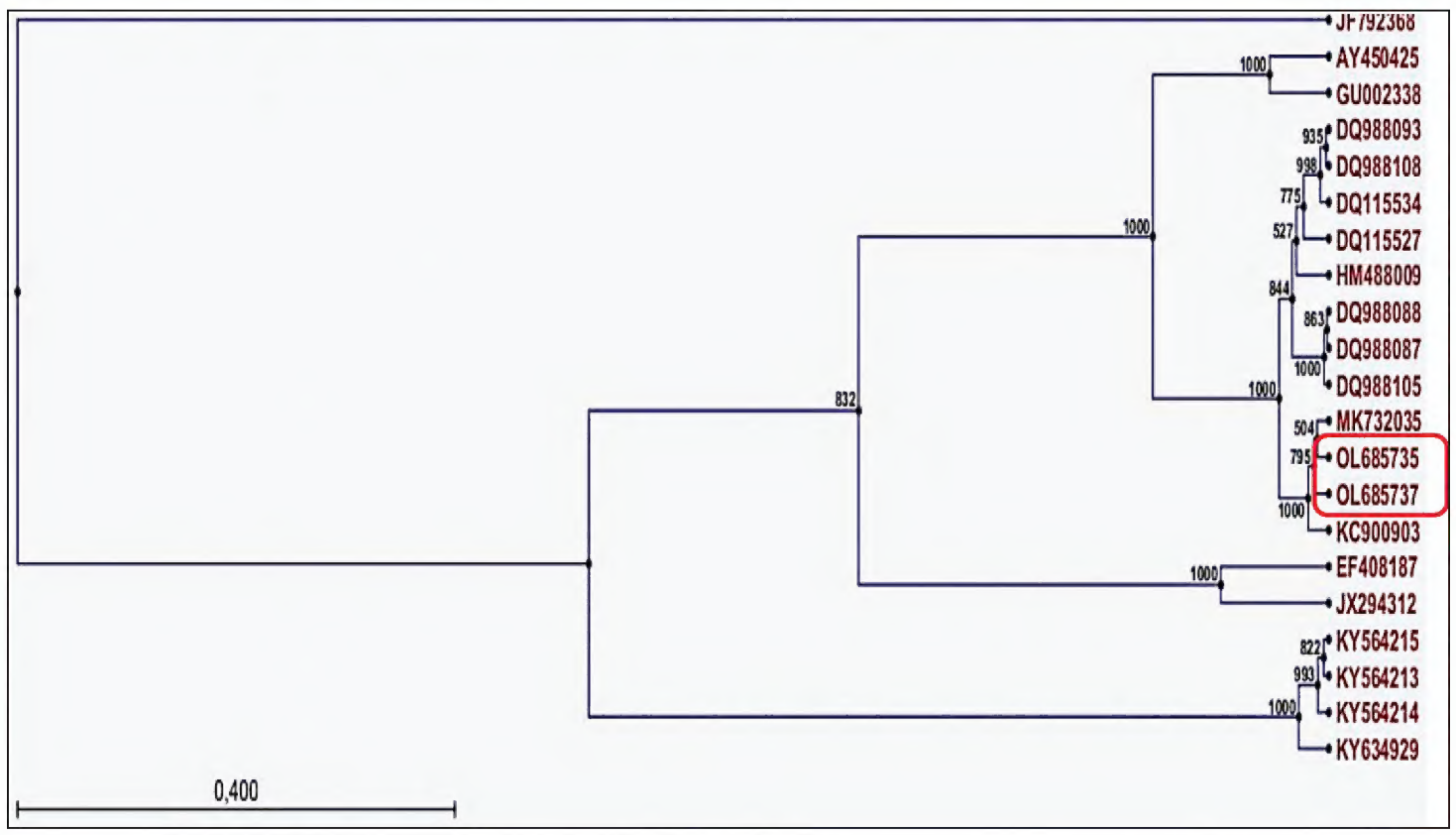

Fig 5. Consensus tree created by Neighbor-Joining Method using 1000 bootstrap values of CPG of Şırnak 12 and Şırnak 33 CYDV-RPV isolates along with isolates from other countries. Turkey-Şırnak isolates are located in the red circle.

Anatolia Region (Usta et al., 2020; Karaozan and Usta; 2020; Hassan et al., 2018), but not as in the Thrace Region and Aegean Region (İlbağı, 2017; Kılıç et al., 2019). The different prevalence of infection is probably due to environmental and climatic factors, change in the vector-insect population annually, differences in the control of vector-insect-weeds, and changes in grain planted areas. As a result, several BYDV sub-strains exist with varying prevalence in Turkey and will continue to threaten wheat crops.

To control BYDV diseases in wheat crops, their reservoirs and vectored insects must be removed from the grain-grown fields. In literature, it was reported that the transmission of BYDVs has prevalently virus-aphid specificity and is actively transported by a few aphid species such as *Sitobion avenae, Rhopalosiphum padi*, *Schizaphis graminum*, *Macrosiphum avenae* (Ingwell et al., 2014; Abraha, 2020). Further, it was reported that grasses and weeds from Poaceae are also alternative hosts to BYDVs (Ilbağı et al., 2018; 2020). Therefore, BYDVs-management strategies such as pest control and weed-free farming practices should be brought to the fore to interrupt possible viral infections in terms of sustainable wheat farming.

CONCLUSION

Using molecular-based methods, we diagnosed BYDV-PAV and CYDV-RPV in wheat plants during the screenings conducted for 5 wheat viruses in Şırnak province. However, no samples tested positive for BYDV-MAV, -SGV, and -RMV. The study identified both single and dual infections. The infection rates in a total of 441 samples were 9.73% for BYDV-PAV, 0.73% for CYDV-RPV, and 2.91% for BYDV-PAV+CYDV-RPV. Molecular analysis confirmed a high nucleotide sequence consensus of the coat protein genes of the viruses with those of other isolates worldwide. Molecular phylogeny showed that Şırnak-BYDV-PAV and Şırnak-CYDV-RPV isolates were closely related to another Turkish isolate.

ACKNOWLEDGMENT

This paper derived from a Master's Thesis with the title "Molecular Characterizations of Some Virus Isolates and Investigation of Barley Yellow Dwarf Viruses (BYDVs) in the Wheat Production Areas in Şırnak Province by Multiplex RT-PCR" written by Orhan KAPAN, under the supervisor of Dr. Mustafa USTA at the Department of Plant Protection of Agriculture Faculty of Van Yuzuncu Yil University. The study was also supported by a grant from The Research Fund of Van Yuzuncu Yil University (Project no: FYL-2020-8882).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Author contributions

All authors contributed equally to this work.

REFERENCES

Abraha, N. W. 2020. Surveillance of *Barley yellow dwarf virus* (BYDV) in All barley growing areas of Ethiopia. South Asian Res. J. Biol. Appl. Biosci. 2: 54-64.

Áy, Z., A. Takács, M. Papp, M. Petróczil, R. Gáborjányi, D. Silhavy, J. Pauk and Z. Kertész. 2008. Detection of cereal viruses in wheat (*Triticum aestivum* L.) by serological and molecular

methods. Cereal Res. Commun. 36: 215-224.

Bashir, M., L. Bertschinger, N. S. Kisana, M. Y. Mujahid and N. I. Hashmi. 1997. Detection of *five barley yellow dwarf luteovirus* serotypes in Pakistan. Rachis. 16: 47-49.

Bisnieks, M., A. Kvarnheden, R. Sigvald and J. P. T. Valkonen. 2004. Molecular diversity of the coat protein-encoding region of *Barley yellow dwarf virus*-PAV and *Barley yellow dwarf virus*-MAV from Latvia and Sweden. Arch. Virol. 149: 843-853.

Bora, T. and İ. Karaca. 1970. Measuring Disease and Damage in Culture Plants. Ege University No: 167, Bornova, p. 43.

Cating, R. A., C. N. Funke, N. Kaur, P. B. Hamm and K. E. Frost. 2015. A multiplex reverse transcription (RT) high-fidelity PCR protocol for the detection of six viruses that cause potato tuber necrosis. Am. J. Pot. Res. 92: 536-540.

Conti, M. C., C. J. D'Arcy, H. Jedlinski and P. A. Burnett. 1990. The "Yellow Plague" of cereals, *barley yellow dwarf*. In: Burnett, P. A. editor. World Perspectives on *Barley yellow dwarf*. CIMMYT, Mexico City D.F, p. 1-6.

D'Arcy, C. J., A. D. Hewings and C. E. Eastman. 1992. Reliable detection of *Barley yellow dwarf viruses* in field samples by monoclonal antibodies. Plant Dis. 76: 273-276.

Deb, M., and J. M. Anderson. 2008. Development of a multiplexed PCR detection method for Barley and *Cereal yellow dwarf viruses*, Wheat spindle streak virus, Wheat streak mosaic virus and *Soil-borne wheat mosaic virus*. J. Virol. Methods. 148: 17-24.

Deligöz, İ., Y. K. Caner and H. Akyol. 2011. Preliminary investigations of *Barley yellow dwarf virus*-PAV and *Barley yellow dwarf virus*-MAV in Wheat fields of samsun and amasya provinces in Turkey. Plant Prot. Bull. 51: 187-193.

Delmiglio, C., M. N. Pearson, R. A. Lister and P. L. Guy. 2010. Incidence of cereal and pasture viruses in New Zealand's native grasses. Ann. Appl. Biol. 157: 25-36.

Diaz, C., V. Saliba-Colombani, O. Loudet, P. Belluomo, L. Moreau, F. Daniel-Vedele, J. F. Morot-Gaudry and C. Masclaux-Daubresse. 2006. Leaf yellowing and anthocyanin accumulation are two genetically independent strategies in response to nitrogen limitation in *Arabidopsis thaliana*. Plant Cell Physiol. 47: 74-83.

El-Yamani, M., and J. H. Hill. 1990. Identification and importance of *Barley yellow dwarf virus in Morocco*. Plant Dis. 74: 291-294.

Erkan, E., and N. D. K. Yılmaz. 2009. Determination of virus diseases on wheat growing areas of Samsun province. Anadolu J. Agric. Sci. 24: 67-75.

Erler, Ö. 2019. Determination of Cereal Viruses in Wheat Growing Areas in Bursa Province. Namık Kemal University, Institute of Science, Tekirdağ.

Foissac, L., P. Gentit, L. Svanetia-Dumas, M. J. Dulucq and T. Candresse. 2001. Polyvalent detection of fruit tree tricho, capillo, and foveaviruses by nested RTPCR using degenerated and inosine containing primers (PDO RT-PCR). Acta Hort. 550: 37-43.

Gambino, G., and I. Gribaudo. 2006. Simultaneous detection of nine grapevine viruses by multiplex reverse transcription-polymerase chain reaction with coamplification of a plant RNA as internal control. Phytopathology. 96: 1223-1229.

Gitton, F., A. Diao, O. Ducrot, J. F. Antoniw, M. J. Adams and H. Maraite. 2002. A two-step multiplex RT-PCR method for simultaneous detection of *soil-borne wheat mosaic virus* and *wheat spindle streak mosaic virus* from France. Plant Pathol. 48: 635-641.

Gomes, C., R. Costa, A. S. Almeida, J. Coutinho, N. Pinheiro, J. Coco, A. Costa and B. Maçãs. 2016. Septoria leaf blotch and yellow rust control by: Fungicide application opportunity and genetic response of bread wheat varieties. Emir. J. Food Agric. 28: 493-500.

Gould, F. W., and R. B. Shaw. 1983. Grass systematics. Brittonia. 35: 301-301.

Gunay, A., and M. Usta. 2020. First investigation of five tobacco viruses using PCR based methods in tobacco plants grown in Adiyaman, Turkey. Fresenius Environ. Bull. 29: 11624-11632.

Hamdi, I., A. Najar, H. B. Ghanem, A. Varsani and A. Jemmali. 2020. First report of *Barley yellow dwarf virus* in maize in Tunisia. Plant Pathol. 102: 227-227.

Hassan, N. O., M. Usta, M. E. Al-Maaroof and A. Güller. 2018. The incidence of *Barley yellow dwarf viruses* (BYDVs) in Wheat Crops in Diyarbakir (Turkey) and Sequence Characterization of BYDV-PAV. YYU J Agric. Sci. 28: 140-149.

Hoffman, T. K., and F. L. Kolb. 1997. Effects of *Barley yellow dwarf virus* on root and shoot growth of winter wheat seedlings grown in aeroponic culture. Plant Dis. 81: 497-500.

İlbağı, H. 2006. Common reed (*Phragmites communis*) is a natural host of important cereal viruses in the Trakya region of Turkey. Phytoparasitica. 34: 441-448.

İlbağı, H. 2017. Epidemic of *yellow dwarf virus* diseases in cereal growing areas and their control. Plant Prot. Bull. 57: 317-335.

İlbağı, H., A. Citir and U. Yorgancı. 2005. Occurrence of virus infections on cereal crops and their identifications in the Trakya region of Turkey. J. Plant Dis. Prot. 112: 313-320.

Ilbagi, H., A. Citir, A. Kara and M. Uysal. 2018. Poaceae weed hosts of *Yellow dwarf viruses* (YDVs) in the Trakya region of Turkey. Ekin. J. 4: 8-19.

Ilbağı, H., A. Çıtır, A. Kara and M. Uysal. 2020. Molecular characterization of cereal *yellow dwarf virus*-RPV in grasses in european part of Turkey. Agriculture (Poľnohospodárstvo). 66: 161-170.

İlbağı, H., F. Rabenstein, A. Habekuss, F. Ordon and A. Çıtır. 2006. Incidence of virus diseases in maize fields in the Trakya Region of Turkey. Phytoprotection. 87: 115-122.

Ingwell, L. L., R. Zemetra, C. Mallory-Smith and N. A. Bosque-Pérez. 2014. Arundo donax infection with *Barley yellow dwarf virus* has implications for biofuel production and non-managed habitats. Biomass Bioenergy. 66: 426-433.

Irwin, M. E., and J. M. Thresh. 1992. BYDV epidemiology: A study in ecological complexity. In: Comeau, A., Makkouk, K. (Eds.). BYDV in West Asia and North Africa. Sayce Publishing, Exeter, UK, p. 3-30.

Jain, N., P. Ramya, H. Krishna, B. Ammasidha, K. C. P. T Kumar, N. Rai, L. Todkar, P. Vijay, M. Pandey, A. Kumar, K. Bisht, K. T. Ramya, V. Jadon, S. Datta, P. K. Singh, G. P. Singh and K. V. Prabhu. 2013. Genomic approaches for improvement of drought and heat in wheat. In: Recent Trends on Production Strategies of Wheat in India. p. 31-37.

Karaozan, A., and M. Usta. 2020. Concurrent detection of five *Yellow dwarf viruses*(B/CYDVs)in wheat in Mardin (Turkey) and phylogeneticrelationship of BYDV-PAV. Not. Bot. Horti. Agrobot. Cluj Napoca. 48: 1862-1872.

Kendall, D. A., S. George and B. D.Smith. 1996. Occurrence of *Barley yellow dwarf viruses* in some common grasses (Gramineae) in South West England. Plant Pathol. 45: 29-37.

Kennedy, T. F., and J. Connery. 2005. Grain yield reductions in spring barley due to *barley yellow dwarf virus* and aphid feeding. Irish J. Agric. Food Res. 44: 111-128.

Khine, M., B. Michaela, Y. Liu, J. Kundu and X. Wang. 2020. Molecular diversity of *barley yellow dwarf virus*-PAV from China and the Czech Republic. J. Integr. Agric. 19: 2736-2745.

Kılıç, H. Ç., D. Altındal, N. Yardımcı and İ. Akgün. 2012. Detection of *Wheat streak mosaic virus* and *Barley stripe mosaic virus* by DAS-ELISA Methods in seeds of different wheat cultivars. J. Agric. Facul. Uludag Univ. 26: 17-25.

Kılıç, H. Ç., N. Yardımcı and D. Toman. 2019. Detection of *Barley yellow dwarf virus*-PAV and *Barley yellow dwarf virus*-MAV in wheat production areas of Isparta and Afyonkarahisar provinces. Anadolu J. Agric. Sci. 34: 129-130.

Köklü, G. 2004. Occurrence of cereal viruses on wheat in Tekirdag, Turkey. Phytoprotection. 85: 19-25.

Kumar, J., J. Ahmad, M. Imtiaz and S. F. Kianian. 2020. Wheat dwarf India Virus and associated betasatellite infecting wheat in Pakistan. Australas. Plant Dis. Notes. 15: 16.

Kumari, S. G., I. Muharram, K. M. Makkouk, A. Al-Ansi, R. El-Pasha, W. A. Al-Motwkel and A. H. Kassem. 2006, Identification of viral diseases affecting barley and bread wheat crops in Yemen. Australas Plant Pathol. 35: 563-568.

Kundu, J. K. 2008. First report of *Barley yellow dwarf virus-PAS* in wheat and barley grown in the czech republic. Plant Dis. 92: 1587.

Kwak, H. R., M. K. Kim, J. C. Shin, Y. J. Lee, J. K. Seo, H. U. Lee, M. N. Jung, S. H. Kim and H. S. Choi. 2014. The current incidence of viral disease in Korean sweet potatoes and development of multiplex RT-PCR assays for simultaneous detection of eight sweet potato viruses. Plant Pathol. J. 30: 416-424.

Kwon, J. Y., J. S. Hong, M. J. Kim, S. H. Choi, B. E. Min, E. G. Song, H. H. Kim and K. H. Ryu. 2014. Simultaneous multiplex PCR detection of seven cucurbit-infecting viruses. J. Virol. Methods. 206: 133-139.

Ibağı, H., F. Rabenstein, A. Habekuss, F. Ordon, A. Çıtır and O. Cececi. 2008. Molecular, serological and tranmission electron microscopic analysis of the *Barley yellow dwarf virus*-PAV and *Cereal yellow dwarf virus*-RPV in Canary Seed (*Phalaris canariensis* L.). Cereal Res. Commun. 36: 225-234.

Li, N., Z. Chen, Y. Liu, Y. Liu, X. Zhou and J. Wu. 2015. Development of monoclonal antibodies and serological assays specific for *Barley yellow dwarf virus* GAV strain. Virol. J. 12: 136.

Malmstrom, C. M., and R. Shu. 2004. Multiplexed RT-PCR for streamlined detection and separation of barley and cereal *yellow dwarf viruses*. J. Virol. Methods. 120: 69-78.

Miller, W. A., and L. Rasochova. 1997. *Barley yellow dwarf viruses*. Ann. Rev. Phytopathol. 35: 167-190.

Nancarrow, N., M. Aftab, G. Hollaway, B. Rodoni and P. Trebicki. 2021. Yield losses caused by *Barley yellow dwarf virus*-PAV infection in wheat and barley: A three-year field study in South-Eastern Australia. Microorganisms. 9: 645.

Nie, X. H., and R. P. Singh. 2000. Detection of multiple potato viruses using an oligo(dT) as a common cDNA primer in multiplex RT-PCR. J. Virol. Methods. 86: 179-185.

Parizoto, G., A. Rebonatto, J. Schons and D. Lau. 2013. *Barley yellow dwarf virus*-PAV in Brazil: Seasonal fluctuation and biological characteristics. Trop. Plant Pathol. 38: 11-19.

Park, K. S., Y. J. Bae, E. J. Jung and S. J. Kang. 2005. RT-PCR-based detection of six garlic viruses and their phylogenetic relationships. J. Microbiol. Biotechnol. 15: 1110-1114.

Perry, K., F. Kolb, B. Sammons, C. Lawson and H. Ohm. 2000. Yiels effects of BYDV in soft red winter wheat. Phytopathology. 90: 1043-1048.

Pocsai, E., A. Citir, H. Ilbagi, G. Köklü, K. Korkut, I. Murányi and G. Vida. 2003. Incidence of *Barley yellow dwarf virus*, *Cereal yellow dwarf virus* and *Wheat dwarf virus* in cereal growing areas of Turkey. Agriculture. 49: 583-591.

Rastgou, M., B. Khatabi, K. Izadpanah, A. R. Afsharifar and M. Masumi. 2005. Relationships of *Barley yellow dwarf virus*-PAV and cereal yellow. Eur. J. Plant Pathol. 113: 321-326.

Roy, A., A. Fayad, G. Barthe and R. H. Brlansky. 2005. A multiplex polymerase chain reaction method for reliable, sensitive and simultaneous detection of multiple viruses in citrus trees. J. Virol. Methods. 129: 47-55.

Saade, M., F. Aparicio, J. A. Sanchez-Navarro, M. C. Herranz, A. Myrta, B. Di Terlizzi and V. Pallas. 2000. Simultaneous detection of the three ilarviruses affecting stone fruit trees by nonisotopic molecular hybridization and multiplex reverse-transcription polymerase chain reaction. Phytopathology. 90: 1330-1336.

Sánchez-Navarro, J. A., F. Aparicio, M. C. Herranz, A. Minafra, A. Myrta and V. Pállas. 2005. Simultaneous detection and identification of eight stone fruit viruses by one-step RT-PCR. Eur. J. Plant Pathol. 111: 77-84.

Shepard J. F., and T. W. Carroll. 1967. Electron microscopy of wheat streak mosaic virus particles in infected plant cells. J. Ultrastruct. Res. 21: 145-152.

Shewry, R. P. 2009. Wheat. J. Exp. Bot. 60: 1537-1553.

Siddiqui, N. N., M. Ilyas, S. Mansoor, A. Azhar and M. Saeed. 2011. Cloning and phylogenetic analysis of coat protein of *Barley yellow dwarf virus* isolates from different regions of Pakistan. J. Phytopathol. 160: 13-18.

Steyer, S., E. Demonty, M. Collard, A. Devaux, Y. Vancoppenolle and C. Bragard. 2005. Detection of BYDV by RT-PCR on single aphids in cereals and maize in Belgium. Parasitica. 61: 37-40.

Tao, Y., J. Man and Y. Wu. 2012. Development of a multiplex polymerase chain reaction for simultaneous detection of wheat viruses and a Phytoplasma in China. Arch. Virol. 157: 1261-1267.

Thackray, D. J., A. J. Diggle and R. A. C. Jones. 2009. BYDY predictor: A simulation model to predict aphid arrival, epidemics of *Barley yellow dwrf virus* and yield losses in wheat crops in a Mediterranean-type environment. Plant Pathol. 58: 186-202.

Trzmiel, K. 2017. Identification of *Barley yellow dwarf viruses* in Poland. J. Plant Pathol. 99: 493-497.

TUIK. 2020. Herbal Product Statistics. Available from: https://data.tuik.gov.tr/bulten/index?p=bitkiseluretim-istatistikleri-2020 [Last accesed on 2021 May 20].

Usta, M., H. M. Sipahioğlu and A. Güller. 2020. Incidence and molecular characterisation of viruses infecting wheat in Eastern Anatolia (Turkey). Fresenius Environ. Bull. 29: 7257-7266.

Webby, G. N., R. M. Lister and P. A. Burnet. 1993. The occurrence of barley yellow dwarf viruses in CIMMYT bread wheat nurseries and associated cereal crops during 1988-1990. Ann. Appl. Biol. 123: 63-74.

Wiese, M. V. 1987. Compendium of Wheat Diseases. 2nd ed. APS Press, St Paul, MN, USA, p. 112.

Yount, D. J., J. M. Martin, T. W. Carroll and S. K. Zaske. 1985. Effects of *Barley yellow dwarf virus* on growth and yield of small grains in Montana. Plant Dis. 69: 487-491.

Zhang, B., T. Thornburg, J. Liu, H. Xue, J. Fontana, G. Wang, Q. Li, K. Davis, Z. Zhang, M. Liu and X. Pan. 2020. Potassium deficiency significantly affected plant growth and development as well as microRNA-mediated mechanism in wheat (*Triticum aestivum* L.). Front. Plant Sci. 11: 1219.

Zhao, X., X. Liu, B. Ge, M. Li and B. Hong. 2015. A multiplex RT-PCR for simultaneous detection and identification of five viruses and two viroids infecting *Chrysanthemum*. Arch. Virol. 160: 1145-1152.